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6. AUTHOR(S)

Robert A. Moss

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Department of Chemistry
Kutgers, The State University of New Jersey
New Brunswick, New Jersey 089038. PERFORMING ORGANIZATION
REPORT NUMBER

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12a. DISTRIBUTION/AVAILABILITY STATEMENT

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12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

We discuss the relationship between the molecular structures of various iodosocarboxylates and their catalytic potency in the cleavages of toxic phosphates and simulants. Iodobenzoate catalysts were successfully and usefully immobilized on silica, titania, nylon, and resin supports. Vesicles and liposomes were utilized as chemical "microreactors". We also studied the dynamic stability of lipids in surface differentiated bilayer liposomes, relating the observed behavior to the molecular structure of the lipids.

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FINAL REPORT

FUNCTIONAL MICELLES AND VESICLES AS ORGANIC REAGENTS

Robert A. Moss

May 15, 1992

U.S. ARMY RESEARCH OFFICE

Army Contract: DAAL03-88-K-0188

Department of Chemistry
Rutgers, The State University of New Jersey
New Brunswick, New Jersey 08903

Attention: Dr. R.G. Chirardelli
Director, Chemical and Biological Sciences Division

Dr. R.P. Seiders
Chief, Colloid and Applied Chemistry

U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709

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A. Statement of the Problem

The overall scientific objective was the development of functional surfactant micelles and vesicles as reaction specific catalysts. The two principal areas of study were: (1) the synthesis of iodosocarboxylate catalysts for the decontamination of toxic phosphates, and (2) the use of vesicles as microreactors.

B. List of Publications: August, 1988 to May, 1992

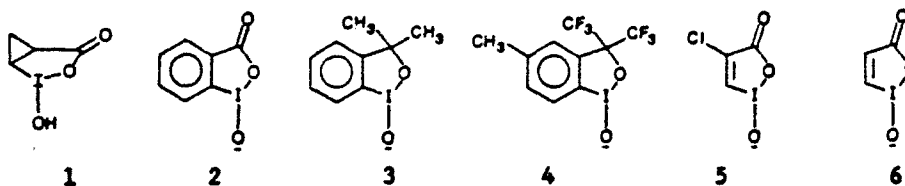
1. "Transvesicular Reactions of Thiols with Ellman's Reagent," R.A. Moss and S. Swarup, J. Org. Chem., 53, 5860 (1988).
2. "Organoiodinane Reagents for Phosphate Cleavage: Experimental and Computational Studies," R.A. Moss, B. Wilk, K. Krogh-Jespersen, J.T. Blair, and J.D. Westbrook, J. Am. Chem. Soc., 111, 250 (1989).
3. "Chemical Differentiation of Bilayer Surfaces in Functional Dialkylammonium Ion Vesicles; Observation of Surfactant Flip-Flop," R.A. Moss, S. Bhattacharya, and S. Chatterjee, J. Am. Chem. Soc., 111, 3680 (1989).
4. "Silica Functionalized with Iodosobenzoate for the Catalytic Cleavage of Reactive Phosphates," R.A. Moss, Y.-C. Chung, H.D. Durst, and J.W. Hovanec, J. Chem. Soc., Perkin Trans. I. (Perkin Commun.), 1350 (1989).
5. "Iodosobenzoate-Functionalized Surfactant Vesicles: Adjustable Reactivity in Reactive Phosphate Cleavage," R.A. Moss and S. Ganguli, Tetrahedron Lett., 30, 2071 (1989).
6. "Synthesis and Properties of the Valence Tautomer of cis-Iodosocyclopropanecarboxylic Acid; 4,5 Methano-1-hydroxyiodoxol-3(1H)-one," R.A. Moss, B. Wilk, K. Krogh-Jespersen, and J.D. Westbrook, J. Am. Chem. Soc., 111, 6729 (1989).
7. "Imidazole Mediated Acylation of Cholesterol in Functional Vesicles: A Simple Analogue of Lecithin:Cholesterol Acyltransferase," R.A. Moss, S. Bhattacharya, and Y. Okumura, Tetrahedron Lett., 30, 4905 (1989).
8. "Surface Specific Cleavage of Synthetic Chromogenic Phospholipid Vesicles," S. Swarup, R.A. Moss, and S. Bhattacharya, "Surfactants in Solution," Vol. 7, K.L. Mittal, Ed., Plenum, New York, 1989, pp. 257-264.

9. "Binding and Transport of Fluorescent Probes in Surfactant Bilayers," S. Swarup and R.A. Moss, "Surfactants in Solution," Vol. 7, K.L. Mittal, Ed., Plenum, New York, 1989, pp. 245-256.
10. "Selective Acetylation of Sterols in Imidazole-Functionalized Surfactant Vesicles," R. A. Moss and Y. Okumura, Tetrahedron Lett., **30**, 5849 (1989).
11. "Immobilized Iodosobenzoate Catalysts for the Cleavage of Reactive Phosphates," R.A. Moss and Y-C. Chung J. Org. Chem. **55**, 2064 (1990).
12. "Proton/Hydroxide Permeation Across Ammonium Ion Bilayer Vesicles Detected by Self-Indicating Head Groups", R.A. Moss and T. Fujita, Tetrahedron Lett., **31**, 2377 (1990).
13. "Dynamics of Surface Differentiated Alkylammonium Ion Vesicles," R.A. Moss, T. Fujita, and S. Ganguli, Langmuir, **6**, 1197 (1990).
14. "Relation of Surfactant Monomer Structure to Flip-Flop Dynamics in Surface-Differentiated Synthetic Bilayer Membranes," R.A. Moss, S. Ganguli, Y. Okumura, and T. Fujita, J. Am. Chem. Soc., **112**, 6391 (1990).
15. "An Efficient Iodosobenzoate-Functionalized Polymer for the Cleavage of Reactive Phosphates," R.A. Moss and Y-C. Chung, Langmuir, **6**, 1614 (1990).
16. "Dynamics of Liposomes Constructed from Phytanyl Lipids," R.A. Moss and T. Fujita, Tetrahedron Lett., **31**, 7559 (1990).
17. "Effect of Unsaturation on Lipid Dynamics within Synthetic Lipid Membranes," R.A. Moss, T. Fujita, and Y. Okumura, Langmuir, **7**, 440 (1991).
18. "Some Kinetic Properties of Mixed Chain Alkylammonium Ion Vesicles," T. Fujita and R.A. Moss, Chem. Lett., 795 (1991).
19. "Dynamics of a Bolaamphiphilic Lipid in a Bilayer Liposome," R.A. Moss, T. Fujita, and Y. Okumura, Langmuir, **7**, 2415 (1991).
20. "Surface Differentiated Model Phospholipid Bilayers," R.A. Moss and Y. Okumura, J. Am. Chem. Soc., **114**, 1750 (1992).

C. Summary of the Most Important Results*

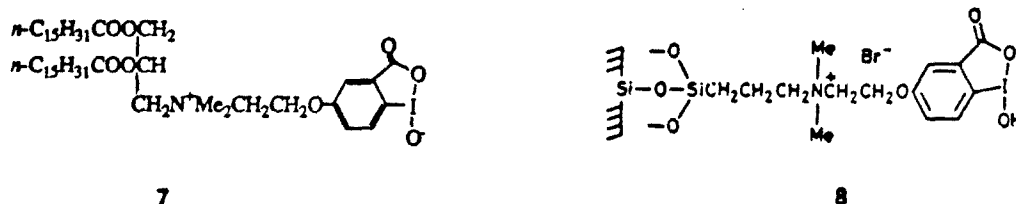
(1) Iodosocarboxylate Catalysts. The valence tautomer of cis-iodosocyclopropane carboxylic acid, 4,5-methano-1-hydroxyiodoxol-3(1H)-one (1), was synthesized from propionic acid in 6 steps. This reagent exists in the illustrated closed form, and cleaves the simulant, *p*-nitrophenyldiphenyl phosphate (PNPDPP), with $k = 0.0044 \text{ s}^{-1}$ in pH 8 aqueous micellar solution.⁶

*Reference superscripts refer to the publications listed in Part B, above.



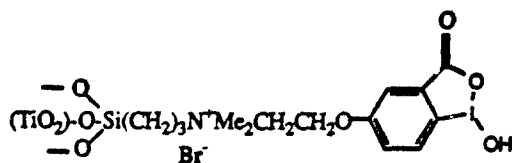
Several analogues of 1-oxido-1,2-benziodoxol-3(1H)-one, 2 (the valence tautomer of *o*-iodosobenzoate), were examined for their ability to cleave PNPDP in aqueous micellar cetyltrimethylammonium chloride (CTACl) at pH 8.² These included the 5,5-dimethyl (3) and 5,5-bis(trifluoromethyl) (4) analogues, as well as the parent 1-oxidoiodoxol-(3H)-ones, 5 and 6. The kinetic reactivity order was $2 > 5 > 6 > 4 \gg 3$. The results were analyzed in terms of the relative acidities of the I-OH forms of the catalysts, and the nucleophilicities of their I-O⁻ conjugate bases. Ab initio molecular orbital calculations aided the analysis.

Covesicles functionalized with iodosobenzoate were constructed from 33% surfactant 7 and 66% dihexadecyldimethylammonium bromide.³ Their kinetic reactivity toward PNPDP at pH 8 was 75 M⁻¹s⁻¹, based on 7. The covesicles were catalytic, turning over in the presence of excess PNPDP.

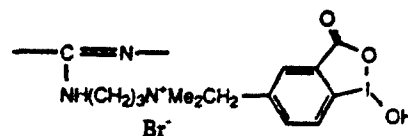


We also prepared several immobilized iodosobenzoate catalysts on silica, titania, nylon, and polymer supports. The silica functionalized decontaminant, 8, was phosphorolytically active against both PNPDP and the nerve agent, soman (GD).⁴ For example, the cleavage of 5 μ mol of GD by 100 mg (10 μ equiv of I-O⁻) of 8 had a half-time of 4.2 min at pH 8, vs. 67 min in the absence of 8.⁴

The iodosobenzoate residue was also immobilized on titania (9) and nylon-6 (10) supports.¹¹ Both 9 and 10 were good catalysts for the cleavage of PNPDP under heterogeneous aqueous conditions at pH 8. The kinetics of cleavage were characterized in the presence of excess substrate (turnover), and in the presence of dilute CTACl, which enhanced both the PNPDP cleavage and catalyst turnover.

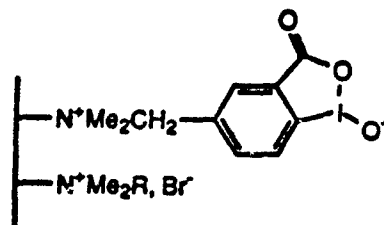


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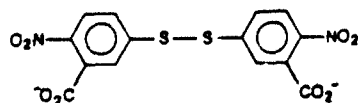
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Finally, the cross-linked macroreticular, acrylic anion-exchange resin IRA-35 was readily converted to the iodosobenzoate/hexadecylammonium-functionalized polymer 11.¹⁵ In pH 8 buffer, 11, catalyzed the cleavage of *p*-nitrophenyldiphenyl phosphate with $k_p = 0.067 \text{ s}^{-1}$, a rate constant comparable to that obtained with iodosobenzoate in CTACl micellar solution. Catalyst 11, turned over when saturated with substrate, with $k_{\text{turn}} = 0.025 \text{ s}^{-1}$ at pH 8. A "family" of functional polymers related to 11 was described.¹⁵

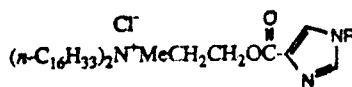


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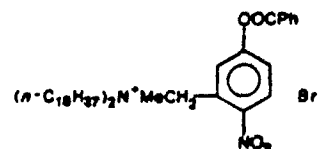
(2) Vesicles as Microreactors. The cleavage of Ellman's reagent, 12, by various thiols was studied in micelles and vesicles at pH 8.¹ Exovesicular and endovesicular cleavage reactions could be kinetically differentiated.



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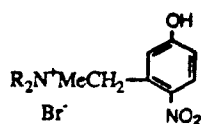


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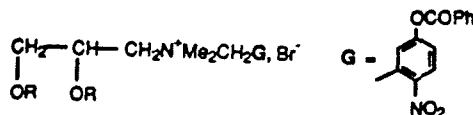
Imidazole-functionalized vesicles of 13 (R-H) were found to transfer acetyl groups from *p*-nitrophenyl acetate (PNPA) to cholesterol within the vesicles.⁷ These reactions, which proceeded via acylimidazole intermediates (13, R-Ac), model the action of the enzyme, lecithin:cholesterol acyltransferase. It was also shown that the acyl transfer reactions were selective: acetyl groups were stereoselectively transferred from PNPA to 3 β in preference to 3 α -cholestanol, and regioselectively transferred to 3 β in preference to 6 β -cholestanol.¹⁰

A major new initiative was the development of a chemical protocol to study the dynamics of lipid "flip-flop" (transvesicular migration) from

endovesicular to exovesicular locations.³ Thus, vesicles of 14, created at pH 4, gave rapid, partial *p*-nitrophenylate cleavage with glutathione at pH 8, due to a surface-specific exovesicular reaction. Flip-flop of intact, endovesicular 14 from endovesicular to exovesicular sites could be promoted and visualized by subsequent experiments involving incubation of the vesicles at 38-40°C for 1-12 min. *p*-Nitrophenol-functionalized vesicular surfactants 15 ($R = n\text{-C}_{16}\text{H}_{33}$, $n\text{-C}_{18}\text{H}_{37}$, or $n\text{-C}_{20}\text{H}_{41}$), which are related to 14, proved to be sensitive, reversible, and continuous reporters of local pH conditions at their endovesicular and exovesicular surfaces.¹²



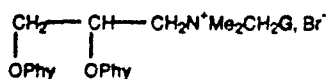
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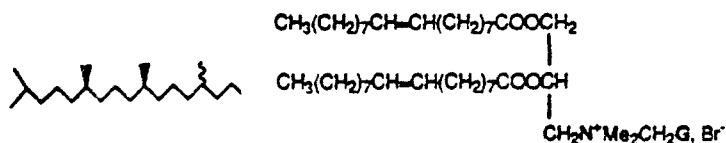
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With a method in hand to study lipid "flip-flop" within vesicular or liposomal membranes,³ we began a broad survey of many new, functional lipids, with the goal of relating each lipid's molecular structure to its flip-flop dynamics within the derived vesicles or liposomes. The effects of surfactant chain length were readily ascertained.^{13,18} In general, longer chain lipids gave liposomes with higher gel to liquid crystal transition temperatures (T_c), and greater thermal stability toward flip-flop.

The survey was next extended to lipids of varying skeletons and backbones. We found that liposomes constructed from pseudoglyceryl lipids (16, $R = n\text{-C}_{16}\text{H}_{33}$ or $n\text{-C}_{15}\text{H}_{31}\text{CO}$) were more resistant to flip-flop than comparably functionalized lipids of the dialkylammonium ion type (e.g., 14)¹⁴ On the other hand, branched chain lipids, as in 17, the bis-phytanyl relative of 16, afforded poorly packed liposomes that featured a very low T_c , and little stability toward flip-flop.¹⁶



17 (G as above)



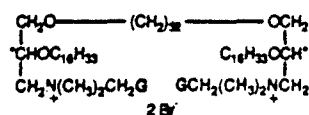
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18 (G as above)

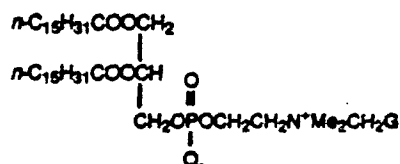
A similar loss of stability was observed with liposomes constructed from unsaturated ammonium ion lipids such as 18.¹⁷ Both the *cis* (oleoyl) and *trans* (elaidoly) modifications of 18 afforded liposomes in which the half-times for

endo → exo lipid flip-flop were much shorter than in the comparable saturated, straight chain, stearyl lipid.

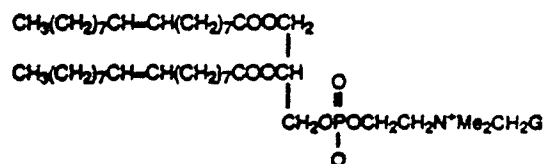
Coliposomes (1:14) of the bolaamphiphilic surfactant 19 and the non-functional host lipid 16 (R-n-C₁₈H₃₃, G-H) were surface differentiated with glutathione.¹⁹ The differentiation kinetics indicated that ~70% of the functional groups of 19 had been in exovesicular locations, whereas ~30% occupied endovesicular sites. Endovesicular/exovesicular flip-flop reequilibration of the differentiated liposomes occurred with $t_{1/2}$ ~ 5 min at 40°C, similar to the behavior of conventional (16) bilayer liposomes. The results suggested that at least 40% of the 19 lipid molecules adopted U-plan conformations in the coliposomes; uniformly extended, bilayer-bridging conformations for the 19 bolaamphiphiles were excluded.



19



20



21

(G as above)

Dipalmitoylphosphatidylcholine 20 (DPPC) and dioleoylphosphatidylcholine 21, (DOPC) lipids were synthesized, in which the head groups were covalently functionalized with p-nitrophenyl benzoate (G) moieties.²⁰ Liposomes composed of 1 part functional lipid and 7 parts nonfunctional DPPC or DOPC were created at pH 6, and subjected to an exovesicular/endovesicular 12/6 pH gradient. Under these conditions, the exovesicular G groups saponified more rapidly ($k \sim 4.5 \times 10^{-2} \text{ s}^{-1}$) than the endovesicular G groups [$k \sim 7 \times 10^{-6} \text{ s}^{-1}$ (DPPC) or $7 \times 10^{-5} \text{ s}^{-1}$ (DOPC)], leading to surface differentiated liposomes with exovesicular p-nitrophenylate labels and intact, endovesicular G groups. The half-times for flip-flop reequilibration of the intact lipids between the endovesicular and exovesicular leaflets of the liposomal bilayers were (DPPC) ~6 min at 65°C and (DOPC) ~20 min at 65°C. Comparisons of DPPC and DOPC flip-flop dynamics to those of analogous, cationic, ammonium ion lipids (such as 18 or 16) highlighted the relative dynamic stability of the zwitterionic phospholipids

in liposomal bilayers.²⁰

In summary, long,¹³ identical,¹⁸ unbranched,¹⁶ saturated¹⁷ alkyl chains on a pseudoglycerol backbone¹⁴ lead to relatively stable endo/exo surface differentiated³ liposomes. The presence of zwitterionic phosphocholine head groups further enhances stability, relative to the analogous cationic lipids.²⁰

Finally, we reviewed the binding and transport of fluorescent probes in bilayer liposomes,⁹ as well as early experiments in the surface specific cleavage of chromogenic phospholipid vesicles.⁸

D. Participating (salaried) Scientific Personnel: 1 Aug 1988 - 31 May 1992

<u>Individual</u>	<u>Title</u>	<u>Period*</u>
Dr. R.A. Moss	Principal Investigator	8/88, 7/89-8/89, 7/90-8/90, 7/91-8/91
Dr. S. Swarup	Post-doctoral Assoc.	8/88-12/88
Mr. S. Ganguli	Graduate Asst.	8/88-8/90
Ms. B. Wilk	Graduate Asst.	8/88-1/89
Ms. S. Chatterjee	Graduate Asst.	8/88-1/89
Ms. H. Zhang	Graduate Asst.	1/89-5/92
Mr. Santanu Bhattacharya	Graduate Asst.	8/88-1/89
Mr. Y-C. Chung	Graduate Asst.	1/89-6/90
Dr. T. Fujita	Post-doctoral Assoc.	5/89-3/91
Mr. Y. Okumura	Graduate Asst.	7/89-8/89, 7/90-8/91
Ms. Z. Dong	Graduate Asst.	7/89-8/89
Ms. Q. Huang	Graduate Asst.	7/89-8/89
Mr. Somendu Bhattacharya	Graduate Asst.	7/90-5/92
Mr. J-M. Li	Graduate Asst.	7/90-8/90, 7/91-5/92
Dr. R. Fujiyama	Post-doctoral Assoc.	8/91-5/92
Dr. G. Li	Post-doctoral Assoc.	8/91-5/92
Mr. C. Yu	Graduate Asst.	7/91-8/91
Ms. H. Zheng	Graduate Asst.	7/91-8/91

*Note: in many instances graduate students were simultaneously appointed as half-time Graduate Assistants and half-time Teaching Assistants.

E. Ph.D. Degrees Awarded

Santanu Bhattacharya, 1989

Swati Chatterjee, 1990

Boguslaw Wilk, 1990

Yong-Chan Chung, 1991

Shovan Ganguli, 1991

Yukihisa Okumura, 1992

F. No inventions were reported